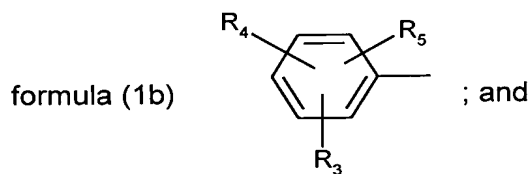
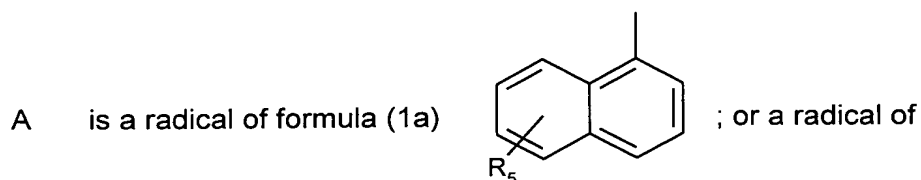
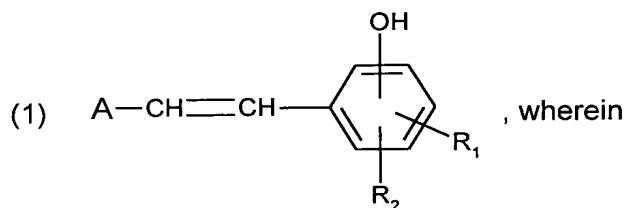


Hydroxystilbene compounds used as microbicidal active substances

The present invention relates to the use of hydroxystilbene compounds in the antimicrobial treatment of surfaces.

The hydroxystilbene compounds used according to the invention correspond to formula



R_1 , R_2 , R_3 , R_4 and R_5 are each independently of the others hydrogen, halogen, hydroxy, C_1 - C_{16} alkyl, C_1 - C_{16} alkoxy, phenyl; C_1 - C_3 phenylalkyl; C_6 - C_{10} aryloxy, amino, mono- C_1 - C_5 alkylamino, di- C_1 - C_5 alkylamino, or $-NO_2$.

C_1 - C_{16} Alkyl are straight-chain or branched alkyl radicals, e.g. methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, amyl, isoamyl or tert-amyl, heptyl, octyl, isooctyl, nonyl, decyl, undecyl, dodecyl, tetradecyl, pentadecyl or hexadecyl.

C_1 - C_{16} Alkoxy is e.g. methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, sec-butoxy, tert-butoxy, amyloxy, isoamyloxy or tert-amyloxy, hexyloxy, heptyloxy, octyloxy, isooctyloxy, nonyloxy, decyloxy, undecyloxy, dodecyloxy, tetradecyloxy, pentadecyloxy or hexadecyloxy.

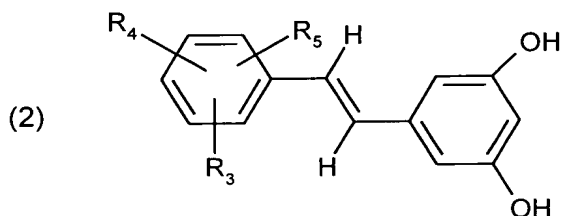
C₆-C₁₀Aryloxy is phenoxy or naphthyloxy.

Halogen is fluorine, chlorine, bromine or iodine.

The hydroxystilbenes used according to the invention can be in the form of E- or Z-isomers. They are preferably in the form of E-isomers.

Interesting compounds that are used according to the invention are dihydroxystilbenes, that is to say compounds of formula (1) wherein R₁ and R₂ are hydroxy.

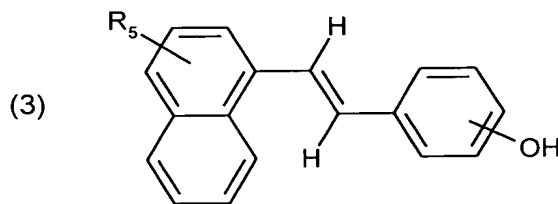
Very special preference is given to the use of compounds of formula



wherein

R₃, R₄ and R₅ are as defined for formula (1), and more especially those compounds of formula (2) wherein R₃, R₄ and R₅ are hydrogen.

Also preferred are compounds of formula



wherein

R₅ is as defined for formula (1) and is especially hydrogen.

The compounds of formula (3) are novel and the invention relates also thereto.

The preparation of the compounds of formula (1) is carried out in accordance with processes known *per se* by reaction of an alkyl phosphite, e.g. triethyl phosphite, with a benzyl halide, preferably benzyl bromide. The phosphonate intermediate is obtained (1st step).

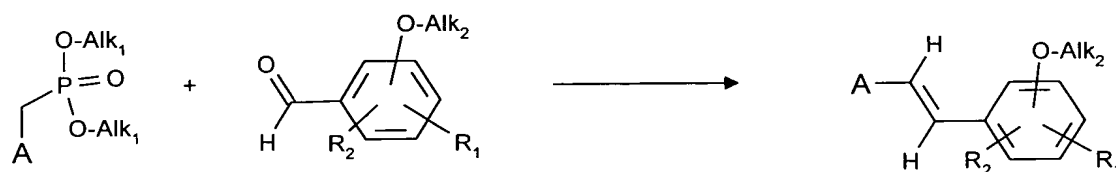
The phosphonate intermediate is then reacted with an alkoxybenzaldehyde (2nd step). The subsequent dealkylation (3rd step) is carried out in accordance with customary methods.

The entire reaction sequence can be illustrated as follows:

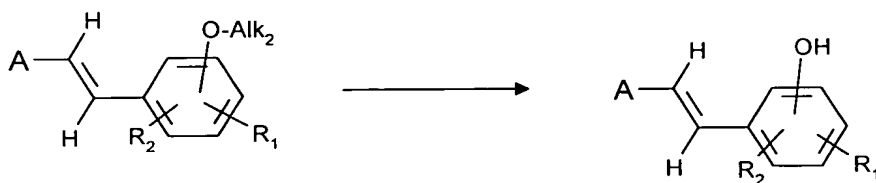
1st step:



2nd step:

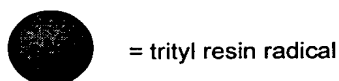
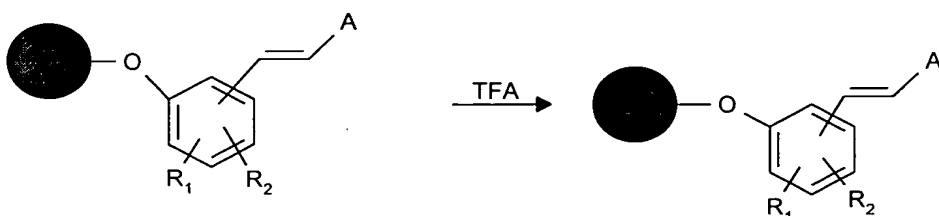
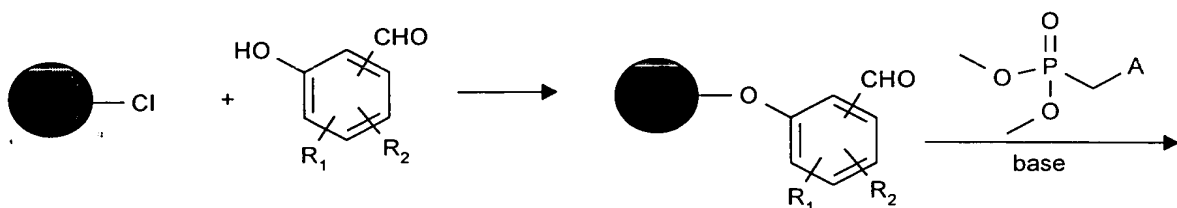


3rd step:



More details relating to this reaction can be found in Can. J. Chem. 48, 1554 (1970).

In a further variant, the hydroxystilbene compounds according to the invention can be prepared in a solid-phase synthesis using a trityl resin. The preparation is carried out in accordance with the following scheme:



wherein R_1 , R_2 and A are as defined for formula (1).

The method of synthesis is based on the literature procedure of R. Willard *et al.*, Chemistry & Biology, 2, 1995, 45-51. The distinguishing feature of the preparation process according to the invention lies in the use of the trityl resin and the different method used for loading the resin.

More details relating to the preparation process according to the invention can be found in the corresponding Examples.

The hydroxystilbene compounds used according to the invention exhibit a pronounced antimicrobial action, especially against pathogenic gram-positive and gram-negative bacteria and also against bacteria of skin flora, e.g. *Corynebacterium xerosis* (bacteria that cause body odour), and also against yeasts and moulds. They are therefore especially suitable in the disinfection of the skin and mucosa and also of integumentary appendages (hair), more especially in the disinfection of the hands and of wounds.

The invention therefore relates also to a personal care preparation comprising at least one compound of formula (1) as well as cosmetically tolerable carriers or adjuvants.

Depending upon the form of the personal care preparation, it will comprise, in addition to the stilbene compound of formula (1), further constituents, for example sequestering agents, colourings, perfume oils, thickening or solidifying agents (consistency regulators), emollients, UV absorbers, skin-protective agents, antioxidants, additives that improve mechanical properties, such as dicarboxylic acids and/or Al, Zn, Ca and Mg salts of C₁₄-C₂₂fatty acids, and optionally preservatives.

The personal care preparation according to the invention may be formulated as a water-in-oil or oil-in-water emulsion, as an alcoholic or alcohol-containing formulation, as a vesicular dispersion of an ionic or non-ionic amphiphilic lipid, as a gel, a solid stick or as an aerosol formulation.

As a water-in-oil or oil-in-water emulsion, the cosmetically tolerable adjuvant contains preferably from 5 to 50 % of an oily phase, from 5 to 20 % of an emulsifier and from 30 to 90 % water. The oily phase may contain any oil suitable for cosmetic formulations, e.g. one or more hydrocarbon oils, a wax, a natural oil, a silicone oil, a fatty acid ester or a fatty alcohol. Preferred mono- or poly-ols are ethanol, isopropanol, propylene glycol, hexylene glycol, glycerol and sorbitol.

Cosmetic formulations according to the invention may be contained in a variety of cosmetic preparations. Especially the following preparations, for example, come into consideration:

- skin-care preparations, e.g. skin-washing and cleansing preparations in the form of tablet-form or liquid soaps, soapless detergents or washing pastes;
- bath preparations, e.g. liquid (foam baths, milks, shower preparations) or solid bath preparations, e.g. bath cubes and bath salts;
- skin-care preparations, e.g. skin emulsions, multi-emulsions or skin oils;
- cosmetic personal care preparations, e.g. facial make-up in the form of day creams or powder creams, face powder (loose or pressed), rouge or cream make-up, eye-care preparations, e.g. eyeshadow preparations, mascara, eyeliner, eye creams or eye-fix creams; lip-care preparations, e.g. lipsticks, lip gloss, lip contour pencils, nail-care preparations, such as nail varnish, nail varnish removers, nail hardeners or cuticle removers;
- intimate hygiene preparations, e.g. intimate washing lotions or intimate sprays;
- foot-care preparations, e.g. foot baths, foot powders, foot creams or foot balsams, special deodorants and antiperspirants or callus-removing preparations;
- light-protective preparations, such as sun milks, lotions, creams and oils, sun blocks or tropicals, pre-tanning preparations or after-sun preparations;
- skin-tanning preparations, e.g. self-tanning creams;
- depigmenting preparations, e.g. preparations for bleaching the skin or skin-lightening preparations;
- insect-repellents, e.g. insect-repellent oils, lotions, sprays or sticks;
- deodorants, such as deodorant sprays, pump-action sprays, deodorant gels, sticks or roll-ons;
- antiperspirants, e.g. antiperspirant sticks, creams or roll-ons;
- preparations for cleansing and caring for blemished skin, e.g. soapless detergents (solid or liquid), peeling or scrub preparations or peeling masks;
- hair-removal preparations in chemical form (depilation), e.g. hair-removing powders, liquid hair-removing preparations, cream- or paste-form hair-removing preparations, hair-removing preparations in gel form or aerosol foams;
- shaving preparations, e.g. shaving soap, foaming shaving creams, non-foaming shaving creams, foams and gels, preshave preparations for dry shaving, aftershaves or after-shave lotions;

- fragrance preparations, e.g. fragrances (eau de Cologne, eau de toilette, eau de parfum, parfum de toilette, perfume), perfume oils or cream perfumes;
- dental-care, denture-care and mouth-care preparations, e.g. toothpastes, gel tooth-pastes, tooth powders, mouthwash concentrates, anti-plaque mouthwashes, denture cleaners or denture fixatives;
- cosmetic hair-treatment preparations, e.g. hair-washing preparations in the form of shampoos and conditioners, hair-care preparations, e.g. pretreatment preparations, hair tonics, styling creams, styling gels, pomades, hair rinses, treatment packs, intensive hair treatments, hair-structuring preparations, e.g. hair-waving preparations for permanent waves (hot wave, mild wave, cold wave), hair-straightening preparations, liquid hair-setting preparations, foams, hairsprays, bleaching preparations, e.g. hydrogen peroxide solutions, lightening shampoos, bleaching creams, bleaching powders, bleaching pastes or oils, temporary, semi-permanent or permanent hair colourants, preparations containing self-oxidising dyes, or natural hair colourants, such as henna or camomile.

An antimicrobial soap has, for example, the following composition:

0.01 to 5 % by weight of the compound of formula (1)

0.3 to 1 % by weight titanium dioxide

1 to 10 % by weight stearic acid

ad 100 % soap base, e.g. the sodium salts of tallow fatty acid and coconut fatty acid or glycerol.

A shampoo has, for example, the following composition:

0.01 to 5 % by weight of the compound of formula (1)

12.0 % by weight sodium laureth-2-sulfate

4.0 % by weight cocamidopropyl betaine

3.0 % by weight NaCl and

water ad 100 %.

A deodorant has, for example, the following composition:

0.01 to 5 % by weight of the compound of formula (1)

60 % by weight ethanol

0.3 % by weight perfume oil and

water ad 100 %.

The invention relates also to an oral composition, comprising from 0.01 to 15 % by weight, based on the total weight of the composition, of the compound of formula (1), and orally tolerable adjuvants.

Example of an oral composition:

10 % by weight sorbitol

10 % by weight glycerol

15 % by weight ethanol

15 % by weight propylene glycol

0.5 % by weight sodium lauryl sulfate

0.25 % by weight sodium methylcocyl taurate

0.25 % by weight polyoxypropylene/polyoxyethylene block copolymer

0.10 % by weight peppermint flavouring

0.1 to 0.5 % by weight of a compound of formula (1) and

48.6 % by weight water.

The oral composition according to the invention may be, for example, in the form of a gel, a paste, a cream or an aqueous preparation (mouthwash).

The oral composition according to the invention may also comprise compounds that release fluoride ions which are effective against the formation of caries, for example inorganic fluoride salts, e.g. sodium, potassium, ammonium or calcium fluoride, or organic fluoride salts, e.g. amine fluorides, which are known under the trade name Olafluor.

The stilbene compounds of formula (1) used according to the invention are also suitable for the treatment of textile fibre materials. Such materials are undyed and dyed or printed fibre materials, e.g. of silk, wool, polyamide or polyurethanes, and especially cellulosic fibre materials of all kinds. Such fibre materials are, for example, natural cellulose fibres, such as cotton, linen, jute and hemp, as well as cellulose and regenerated cellulose. Preferred suitable textile fibre materials are made of cotton.

The stilbene compounds of formula (1) are also used in washing and cleaning formulations, e.g. in liquid and powder washing agents or softeners.

The stilbene compounds used according to the invention are also suitable for the treatment of plastics, especially for imparting antimicrobial properties to or preserving plastics, e.g. polyethylene, polypropylene, polyurethane, polyester, polyamide, polycarbonate, latex etc.. Fields of use therefor are, for example, floor coverings, plastics coatings, plastics container and packaging materials; kitchen and bathroom utensils (e.g. brushes, shower curtains; sponges, bathmats), latex, filter materials (air and water filters), plastics articles used in the field of medicine, e.g. dressing materials, syringes, catheters etc., so-called "medical devices", gloves and mattresses.

Paper, for example papers used for hygiene purposes, may also be provided with antimicrobial properties using the stilbene compounds according to the invention.

It is also possible for nonwovens, e.g. nappies/diapers, sanitary towels, panty liners, and cloths for hygiene and household uses, to be provided with antimicrobial properties in accordance with the invention.

The stilbene compounds can be used especially also in household and all-purpose cleaners for cleaning and disinfecting hard surfaces.

A cleaning preparation has, for example, the following composition:

0.01 to 5 % of the compound of formula (1)
3.0 % octyl alcohol 4EO
1.3 % fatty alcohol C₈-C₁₀polyglucoside
3.0 % isopropanol
ad 100 % water.

In addition to preserving cosmetic and household products, technical products, such as paper treatment liquors, printing thickeners of starch or of cellulose derivatives, surface-coatings and paints, can be preserved and provided with antimicrobial properties.

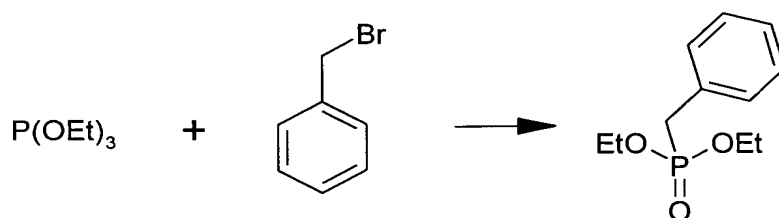
The stilbene compounds of formula (1) are also suitable for the antimicrobial treatment of wood and for the antimicrobial treatment of leather, the antimicrobial preservation of leather and the provision of leather with antimicrobial properties.

The compounds according to the invention are also suitable for the protection of cosmetic products and household products from microbial spoilage.

The following Examples serve to illustrate the invention but do not limit the invention to the Examples.

Example 1: Preparation of 3,5-dihydroxystilbene

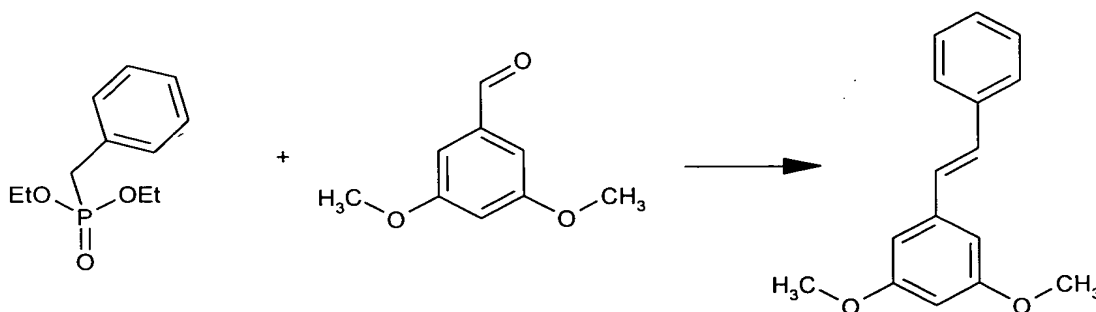
1st step:



A mixture of 51.3 g (0.3 mol) of benzyl bromide and 79.1 g (0.5 mol) of triethyl phosphite is heated at 130°C until the evolution of gas has ceased (3 h). The excess of triethyl phosphite is then removed under a water-jet vacuum. The crude product can be used for the next reaction without further purification.

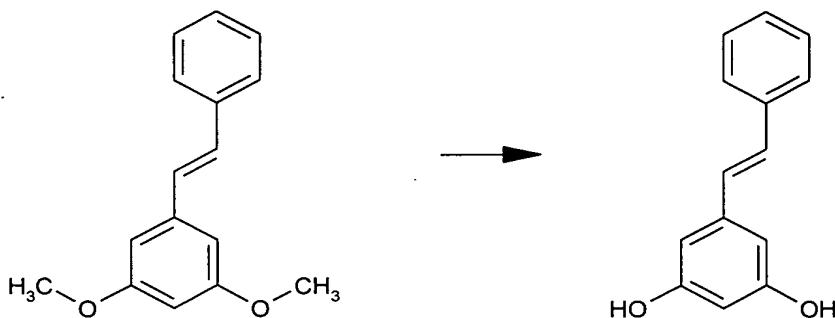
Yield: 60 g (0.29 mol; 96.6 % of theory)

2nd step:



16.5 g (0.3 mol) of sodium methanolate are added at 0°C to a solution of 60 g (0.29 mol) of crude diethylbenzyl phosphonate in 415 ml of anhydrous DMF. Then, at 0°C, a total of 50.0 g (0.3 mol) of 3,5-dimethoxybenzaldehyde is added in portions. After stirring for 1 hour at room temperature and heating for 1 hour under reflux, the product is precipitated by the addition of 660 ml of water/methanol (mixture ratio 2:1). Recrystallisation from water/methanol (2:1) yields 3,5-dimethoxystilbene in the form of colourless crystals. Yield: 54.0 g (0.22 mol, 73.3 % of theory)

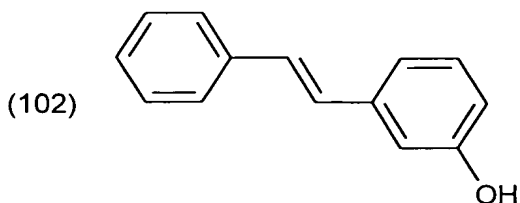
3rd step:



For demethylation, a homogeneous mixture of 54.0 g (0.22 mol) of 3,5-dimethoxystilbene and 40.0 g (0.35 mol) of pyridine hydrochloride is heated at about 165°C for 3 hours. The cooled, oily reaction mass is then introduced into 1.2 litres of 2N hydrochloric acid and the crude product is isolated by extraction with diethyl ether. Recrystallisation from toluene yields 3,5-dihydroxystilbene in the form of a pale-yellow powder. Yield: 26.0 g (0.12 mol; 41.0 % of theory)

Example 2:

Analogously to Example 1, reaction of 20.0 g (0.12 mol) of benzyl bromide, 38.9 g (0.23 mol) of triethyl phosphite and 15.9 g (0.12 mol) of 3-methoxybenzaldehyde yields 7.0 g of 3-hydroxystilbene, corresponding to formula



Example 3: Determination of the minimum inhibiting concentration (MIC) in the agar diffusion test

Medium:	Mueller-Hinton agar (Merck) * Sabouraud 4 % glucose agar (Merck)
Dilution medium:	sterile 0.85 % NaCl solution
Test organisms:	Staphylococcus aureus ATCC 9144 Corynebacterium xerosis ATCC 373 Escherichia coli NCTC 8196 Pseudomonas aeruginosa CIP A-22 Candida albicans ATCC 10231 * Aspergillus niger ATCC 6275
Incubation:	24 hours at 37°C * 3 days at 28°C
Test solution:	5 % stock solutions of all the test substances in a suitable solvent are prepared and diluted in serial dilutions to final concentrations of from 1000 ppm to 10 ppm.
Test principle:	0.3 ml of the dilution stage in question is mixed with 15 ml of still-liquid nutrient medium. When the nutrient substrate has solidified, 10 µl portions of the following organism dilution of the test strains in 0.85 % NaCl solution are spotted onto the agar medium:

Staphylococcus aureus ATCC 9144	1:10 dilution
Corynebacterium xerosis ATCC 373	1:100 dilution
Escherichia coli NCTC 8196	1:100 dilution
Pseudomonas aeruginosa CIP A-22	1:100 dilution
Candida albicans ATCC 10231	1:10 dilution
* Aspergillus niger ATCC 6275	1:10 dilution

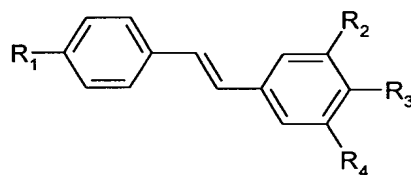
The plates are incubated at 37°C for 24 hours (A.niger 3 days at 28°C) and then the highest dilution of the test substance at which

growth is just no longer discernible (corresponds to MIC) is determined.

The results show that the test substances exhibit strong antimicrobial activity against gram-positive and gram-negative bacteria and also fungi.

The test results for the compounds listed below are given in Table 1:

General formula:



<u>Compound of formula</u>	<u>R₁</u>	<u>R₂</u>	<u>R₃</u>	<u>R₄</u>
(101)	H	OH	H	OH
(102)	H	OH	H	H
(103)	OH	OH	H	OH
(104)	H	H	N(CH ₃) ₂	H
(105)	OH	H	H	H
(106)	OH	H	Cl	H

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Table 1:

Organisms	Compound of formula (101) ¹	Compound of formula (102) ²	Compound of formula (103) ³	Compound of formula (104) ⁴	Compound of formula (105) ⁵	Compound of formula (106) ⁶
S. aureus	100	100	600	300	--	10
C. xerosis	--	--	100	--	--	--
E. coli	100	100	600	--	--	--
P. aeruginosa	1000	--	600	--	--	--
C. albicans	100	100	600	600	--	--
A. niger	100	10	--	--	10	600

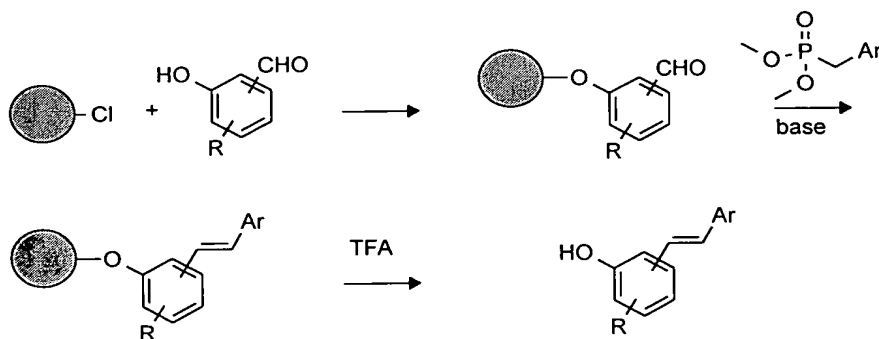
(all values MIC concentrations in ppm)

-- = not tested

Examples 4 to 87: Solid-phase synthesis method

The following hydroxystilbenes are synthesised in accordance with known procedures (R. Willard *et al.*, Chemistry & Biology, 2, 1995, 45-51).

The reaction is carried out in accordance with the following scheme:



¹ solution EtOH

² solution in DMSO

³ solution in EtOH

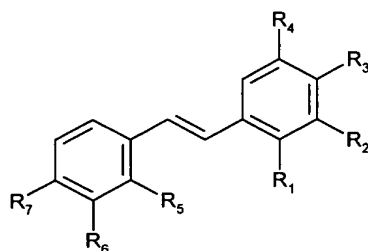
⁴ solution in DMSO

⁵ solution in DMSO


⁶ solution in DMSO

Using this method a matrix of $12 \times 7 = 84$ hydroxystilbenes having the following structural scope is synthesised:












General formula:



Comp. of formula	<u>R₁</u>	<u>R₂</u>	<u>R₃</u>	<u>R₄</u>	<u>R₅</u>	<u>R₆</u>	<u>R₇</u>
107	H	H	OH	H	H	H	H
108	H	OH	H	H	H	H	H
109	H	OH	OMe	H	H	H	H
110	H	OEt	OH	H	H	H	H
111	H	OMe	OH	H	H	H	H
112	OH	H	OMe	H	H	H	H
113	OH	H	H	H	H	H	H
114	H	OH	OMe	OMe	H	H	H
115	H	Me	OH	Me	H	H	H
116	OH	Me	H	H	H	H	H
117	OH	H	OBzl	H	H	H	H
118	OMe	H	OH	H	H	H	H
119	H	H	OH	H	H	H	Cl
120	H	OH	H	H	H	H	Cl
121	H	OH	OMe	H	H	H	Cl
122	H	OEt	OH	H	H	H	Cl
123	H	OMe	OH	H	H	H	Cl
124	OH	H	OMe	H	H	H	Cl
125	OH	H	H	H	H	H	Cl
126	H	OH	OMe	OMe	H	H	Cl

Comp. of formula	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇
127	H	Me	OH	Me	H	H	Cl
128	OH	Me	H	H	H	H	Cl
129	OH	H	OBzl	H	H	H	Cl
130	OMe	H	OH	H	H	H	Cl
131	H	H	OH	H	H	H	OMe
132	H	OH	H	H	H	H	OMe
133	H	OH	OMe	H	H	H	OMe
134	H	OEt	OH	H	H	H	OMe
135	H	OMe	OH	H	H	H	OMe
136	OH	H	OMe	H	H	H	OMe
137	OH	H	H	H	H	H	OMe
138	H	OH	OMe	OMe	H	H	OMe
139	H	Me	OH	Me	H	H	OMe
140	OH	Me	H	H	H	H	OMe
141	OH	H	OBzl	H	H	H	OMe
142	OMe	H	OH	H	H	H	OMe
143	H	H	OH	H	H	H	Ph
145	H	OH	H	H	H	H	Ph
146	H	OH	OMe	H	H	H	Ph
147	H	OEt	OH	H	H	H	Ph
148	H	OMe	OH	H	H	H	Ph
149	OH	H	OMe	H	H	H	Ph
150	OH	H	H	H	H	H	Ph
151	H	OH	OMe	OMe	H	H	Ph
152	H	Me	OH	Me	H	H	Ph
153	OH	Me	H	H	H	H	Ph
154	OH	H	OBzl	H	H	H	Ph
155	OMe	H	OH	H	H	H	Ph
156	H	H	OH	H			H

0906844-04031

Comp. of formula	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇
157	H	OH	H	H			H
158	H	OH	OMe	H			H
159	H	OEt	OH	H			H
160	H	OMe	OH	H			H
161	OH	H	OMe	H			H
162	OH	H	H	H			H
163	H	OH	OMe	OMe			H
164	H	Me	OH	Me			H
165	OH	Me	H	H			H
166	OH	H	OBzl	H			H
167	OMe	H	OH	H			H
168	H	H	OH	H	Me	H	H
169	H	OH	H	H	Me	H	H
170	H	OH	OMe	H	Me	H	H
171	H	OEt	OH	H	Me	H	H
172	H	OMe	OH	H	Me	H	H
173	OH	H	OMe	H	Me	H	H
174	OH	H	H	H	Me	H	H
175	H	OH	OMe	OMe	Me	H	H
176	H	Me	OH	Me	Me	H	H

09806344-040501

<u>Comp. of formula</u>	<u>R₁</u>	<u>R₂</u>	<u>R₃</u>	<u>R₄</u>	<u>R₅</u>	<u>R₆</u>	<u>R₇</u>
177	OH	Me	H	H	Me	H	H
178	OH	H	OBzl	H	Me	H	H
179	OMe	H	OH	H	Me	H	H
180	H	H	OH	H	H	H	Me
181	H	OH	H	H	H	H	Me
182	H	OH	OMe	H	H	H	Me
183	H	OEt	OH	H	H	H	Me
184	H	OMe	OH	H	H	H	Me
185	OH	H	OMe	H	H	H	Me
186	OH	H	H	H	H	H	Me
187	H	OH	OMe	OMe	H	H	Me
188	H	Me	OH	Me	H	H	Me
189	OH	Me	H	H	H	H	Me
190	OH	H	OBzl	H	H	H	Me
191	OMe	H	OH	H	H	H	Me

Me = methyl

Et = ethyl

Bzl = benzyl

The microbiological data obtained are summarised in Table 2.

Table 2: MIC values in ppm for various microorganisms*)

<u>Comp. of formula</u>	<u>S. heminis</u>	<u>E. coli</u>	<u>P. aeruginosa</u>	<u>C. albicans</u>	<u>A. niger</u>
107	100	>100	>100	>100	100
108	60	30	100	30	30
109	>100	>100	>100	>100	100
110	>100	>100	>100	>100	>100
111	>100	>100	>100	>100	100
112	---	---	---	---	---

<u>Comp. of formula</u>	<u>S. heminis</u>	<u>E. coli</u>	<u>P. aeruginosa</u>	<u>C. albicans</u>	<u>A. niger</u>
113'	100	100	>100	60	60
114	>100	100	>100	>100	100
115	---	---	---	---	---
116	60	60	100	60	60
117	>100	>100	>100	>100	100
118	---	---	---	---	---
119	7.5	>100	>100	15	100
120	7.5	7.5	>100	7.5	30
121	>100	>100	>100	>100	>100
122	>100	>100	>100	>100	>100
123	>100	>100	>100	>100	100
124	15	15	>100	30	30
125	60	15	100	60	60
126	>100	>100	>100	100	100
127	---	---	---	---	---
128	>100	>100	>100	>100	100
129	---	---	---	---	---
130	---	---	---	---	---
131	>100	>100	>100	>100	>100
132	>100	>100	>100	>100	>100
133	>100	>100	>100	>100	>100
134	>100	>100	>100	>100	>100
135	>100	>100	>100	>100	>100
136	---	---	---	---	---
137	>100	100	>100	>100	>100
138	>100	>100	>100	>100	>100
139	---	---	---	---	---
140	>100	>100	>100	>100	100
141	---	---	---	---	---

113' 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141

<u>Comp. of formula</u>	<u>S. heminis</u>	<u>E. coli</u>	<u>P. aeruginosa</u>	<u>C. albicans</u>	<u>A. niger</u>
142	---	---	---	---	---
143	>100	>100	>100	>100	>100
145	>100	>100	>100	>100	>100
146	>100	>100	>100	>100	>100
147	>100	>100	>100	>100	>100
148	>100	>100	>100	>100	>100
149	---	---	---	---	---
150	>100	>100	>100	>100	>100
151	>100	>100	>100	>100	>100
152	---	---	---	---	---
153	>100	>100	>100	>100	>100
154	---	---	---	---	---
155	---	---	---	---	---
156	7.5	60	>100	15	30
157	7.5	15	>100	15	60
158	>100	>100	>100	>100	>100
159	>100	>100	>100	>100	100
160	>100	>100	>100	>100	100
161	---	---	---	---	---
162	7.5	30	>100	30	15
163	>100	>100	>100	>100	100
164	---	---	---	---	---
165	100	>100	>100	>100	100
166	---	---	---	---	---
167	---	---	---	---	---
168	7.5	30	100	15	30
169	30	60	100	30	30
170	>100	>100	>100	>100	>100
171	>100	>100	>100	>100	>100

0906344-040501

<u>Comp. of formula</u>	<u>S. heminis</u>	<u>E. coli</u>	<u>P. aeruginosa</u>	<u>C. albicans</u>	<u>A. niger</u>
172	>100	>100	>100	10	>100
173	---	---	---	---	---
174	60	100	>100	60	60
175	60	60	100	60	100
176	---	---	---	---	---
177	30	60	>100	60	60
178	---	---	---	---	---
179	---	---	---	---	---
180	100	>100	>100	>100	>100
181	15	15	100	15	30
182	>100	>100	>100	>100	>100
183	>100	>100	>100	>100	>100
184	>100	>100	>100	>100	100
185	---	---	---	---	---
186	30	30	100	30	60
187	>100	>100	>100	>100	>100
188	---	---	---	---	---
189	>100	100	>100	>100	>100
190	---	---	---	---	---
191	---	---	---	---	---

--- = not determined

*) The MIC values were obtained by measuring the optical density at substance concentrations between 100; 10 and 1 ppm. In that respect some of the data are indicative values for the activity. The MIC values of the compounds having good activity were obtained by measuring the optical density at concentrations between 120; 60; 30; 15; 7.5; 3.75 ppm.

Determination of the minimum inhibiting concentration (MIC value) in microtitre plates:

Nutrient:medium:

Casein/soybean flour peptone bouillon for the preparation of the precultures of the test bacteria and yeast.

Mycological slant agar for the preculture of moulds.

Examples of test organisms:

Bacteria: Staphylococcus hominis DSM 20328

Escherichia coli NCTC 8196

Pseudomonas aeruginosa CIP A-22

Yeast: Candida albicans ATCC 10231

Mould: Aspergillus niger ATCC 6275

Procedure:

The test substances are predissolved in dimethyl sulfoxide (DMSO) and tested in a serial dilution of 1:2.

Bacteria and yeast are cultured overnight in CASO bouillon, the mould on mycological slant agar and rinsed off with 10 ml of 0.85 % sodium chloride solution (+ 0.1 % TritonX-100).

All test organisms are adjusted to an organism count of 1.5×10^6 CFU/ml with 0.85 % sodium chloride solution.

The test substances are prepipetted into microtitre plates in an amount of 8 µl per well.

Previously diluted organism substances are diluted 1:100 in CASO bouillon (bacteria and yeast) and Sabouraud 2 % glucose bouillon (mould) and added to the test substances in an amount of 192 µl per well.

The test batches are incubated for 48 hours at 37°C (bacteria and yeast) or for 5 days at 28°C (mould).

After incubation, the growth is determined by reference to the turbidity of the test batches (optical density) at 620 nm in a microplate reader.

The minimum inhibiting concentration (MIC value) is the concentration of substance at which (compared with the growth control) an appreciable inhibition of the growth ($\leq 20\%$ growth) of the test organisms is ascertained.

One microtitre plate is used for each test organism and substance concentration. All substances are tested in duplicate.

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